a3 16. (Amended) The method of claim 1, wherein the oocyte and the sperm head are from a mammal.[.]

21. (Amended) A method for obtaining a transgenic embryo, comprising the steps of:

obtaining a membrane-disrupted sperm head or a demembranated sperm head;

mixing the membrane-disrupted sperm head or demembranated sperm head with an exogenous nucleic acid containing a desired gene[,];

[coinjecting] microinserting the mixture into an isolated unfertilized metaphase II oocyte to form a transgenic fertilized oocyte; and

allowing the transgenic fertilized oocyte to develop into a transgenic embryo.

REMARKS

Reconsideration of the above-identified application, as amended, is respectfully requested. This amendment is in response to an Office Action dated April 11, 2000.

By this amendment, claim 9 has been canceled and its subject matter incorporated into amended claim 1. Claim 16 has been amended to delete the second period in response to the Examiner's objection. Withdrawal of this objection is respectfully requested. Claims 1-3, 10-12, 16 and 21 have been amended to more distinctly point out certain features of applicants' invention. No new matter has been added.

Claims 1-8 and 10-21 remain pending.

The Rejections Under 35 U.S.C. § 112

The Examiner rejected claims 9-13 under 35 U.S.C. 112, second paragraph, alleging that claims 9-13 contain the term "first time period" which allegedly does not convey any clear meaning. By this amendment, claim 9 has been deleted, and claims 10-12 have been amended to delete "first". Applicants respectfully submit that claim 13 does not contain the term "first time period" making this rejection moot. Applicants submit that the amended claims 9-12 are fully responsive and respectfully request the withdrawal of the rejections under 35 U.S.C. 112, second paragraph.

The Examiner has rejected claims 1-21 under 35 U.S.C. § 112, first paragraph, alleging that the specification, while being enabling for a method of creating a transgenic embryo or a transgenic mouse, does not reasonably provide enablement for a method of obtaining a transgenic embryo or creating transgenic animals, ***of any and all species***. The Examiner further alleges that in view of the unpredictable state of the art, the absence of working examples for the demonstration of production of transgenic animals other than the mouse, and species differences in the expression of transgenes, one skilled in the art would have to undergo undue experimentation to successfully produce transgenic embryos and transgenic live offspring of any and all species according to the method of the invention.

Applicants respectfully traverse this rejection. Applicants submit that the current body of scientific evidence supports the contention that the invention method may be successfully used to produce transgenic embryos and transgenic live offspring "any and all species" without undue experimentation. In particular, the ability of whole live sperm cells to take up exogenous DNA has been demonstrated in a wide variety of animal species, and the exogenous DNA has been reported to become associated with the nucleus in the head of the sperm. Applicants submit herewith, in a Supplemental Information Disclosure Statement, copies of the following newly discovered publications which demonstrate that sperm cells from virtually every species of animal in which the genetic material is transferred to the oocyte via live sperm are capable of acting as vectors for the transmission of DNA. Species that have been shown to demonstrate this capacity include, for example, rabbit, sea urchin, abalone, blow fly, honeybee, zebrafish, *Xenopus laevis*, rooster, mouse, rabbit, ram, goat, pig, buffalo, bull, golden hamster and human. See reviews by Kevin R. Smith (Animal Biotechnology **10**, 1-13, 1999, see, *e.g.,* Tables pages 4-6), Corrado Spadafora (BioEssays **20.11**, 955-964, 1998, see, *e.g.,* Table 1, page 957), Fulvio Gandolfi (Transgenic Research 7, 147-155, 1998, see *e.g.,* Table 1, page 152), and F. Gandolfi (Theriogenology **53**, 127-137, 2000 see *e.g.,* Tables 1 and 2A, pages 128 and 132), and publications by Tsai *et al.*, 1997; Patil and Khoo, 1996; Atkinson, *et al.,* 1991; Magnano *et al.,* 1998; Fernandez, *et al.*, 1999; and Kuznetsov, *et al.*, 1995.

Applicants respectfully assert that the newly discovered art demonstrates that the exogenous DNA can also be transferred to eggs via live sperm during fertilization in a wide variety of species to produce genetically transformed embryos and animals. Species described in the above

publications that have demonstrated the capacity to be fertilized by live sperm carrying exogenous DNA with subsequent embryonic development include sea urchins to the blastulae developmental stage with CAT transgene expression, abalone to the trochofore larvae developmental stage with CAT transgene expression, silkworm to the larvae developmental stage with expression of the CAT transgene, salmon to the fry developmental stage, zebrafish to the adult developmental stage, cat fish to the fry developmental stage with kanamycin phosphotransferase transgene expression, Xenopus laevis to the embryo developmental stage with src transgene expression, rooster to the fetus developmental stage , mouse to the fetus developmental stage with CAT transgene expression, rabbit to the blastocyst developmental stage with LacZ transgene expression, pig to the adult developmental stage, and bovine to the adult developmental stage.

Contrary to the Examiner's allegations, applicants respectfully assert that the publications presented above provide support for the ***equivalency of live sperm from various vertebrate and invertebrate species to take up and transfer exogenous DNA into an oocyte, resulting in the development of transgenic embryos and live transgenic offspring***. Therefore, absent evidence to the contrary by the Examiner, applicants assert the comparable equivalency of membrane-disrupted sperm heads or demembranated sperm heads from vertebrate and invertebrate species to take up and transfer exogenous DNA into oocytes.

Moreover, the publications support the association of the exogenous DNA with the nucleus of the sperm. The present invention differs from all of the above methods of transfer of exogenous DNA in that the sperm are not "live"either during their exposure to the exogenous DNA or their microinsertion into the oocyte. In the present invention, the association of the exogenous DNA with the sperm nucleus occurs in a very short time period during incubation of "dead"membrane-disrupted or demembranated sperm heads (nuclei) with the exogenous DNA. Gandolfi (Theriogenology **53**, 127-137, 2000 at page 131, paragraph 1) refers to applicants' invention and publication in Science (1999) **284**, 1180-1183, as follows: "Recently, an ***innovative*** approach to the production of transgenic mice through sperm-mediated gene transfer was described...This method causes disruption of the sperm membrane and damage of nuclear DNA in the form of single-strand breaks. Both events are likely to facilitate the association of exogenous DNA with nuclear structures which, in turn, stabilizes the transgene within the oocyte, thereby facilitating its integration

in the resulting embryonic genome. Facilitating DNA penetration into sperm cells has been a successful strategy to improve rates of transgenesis in other species...". Moreover, Schatten et al. (*Mol. Hum. Reprod.*, 6(1): 26-33, (2000) reported that, in rhesus monkeys, intracytoplasmic sperm injection (ICSI) with spermatozoa that had been incubated with a plasmid containing a jelly fish green fluorescent protein gene resulted in the production of fluorescent embryos, and a live offspring although there was no evidence that the gene was incorporated into any of the offspring's cells. However, according to an article in the New York Times of December 23, 1999 (copy enclosed), ***Dr. Schatten and other scientists say they have no doubt that the method will work because it was recently shown to work in mice.*** The method to which they are referring is the method of the inventors of the present invention, as published in the Science article.

In view of the foregoing recited publications and the testimonials of respected scientists of ordinary skill in the art, applicants respectfully submit that there is no reason to expect that the method of the invention can not be successfully used for any and all species, including the exemplified mouse, or that such successful use would require undue experimentation by those skilled in the art. Applicants therefore respectfully request the withdrawal of these rejections of claim 1-21 under 35 U.S.C 112, first paragraph.

The Examiner further rejects claims 1-20 under 35 U.S.C. 112, first paragraph, alleging that the claims read on any method of transferring a sperm head and exogenous DNA into an oocyte, and all methods of "co-inserting" of the sperm head and exogenous DNA into the oocyte. By this amendment, applicants have amended the claims to require the step of "incubating an exogenous nucleic acid with a membrane-disrupted sperm head or a demembranated sperm head for a period of time to obtain a sperm head comprising an associated exogenous nucleic acid, prior to the step of microinserting the resulting sperm head into an unfertilized oocyte. Applicants respectfully submit that this amendment fully addresses and overcomes the Examiner's rejection on the basis of "co-insertion". Further, applicants respectfully submit that methods of microinsertion of nuclei into oocytes are well known to those of ordinary skill in the art and do not require undue experimentation. Applicants respectfully submit that the use of piezo electric microinjection is but one of these microinsertion methods, that the exemplary use of piezo electric microinjection in the specification was merely convenient, and that the invention method is not limited to this microinjection method.

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In view of the foregoing, applicants respectfully request withdrawal of these rejections of claims 1-20 under 35 U.S.C. 112, first paragraph.

The Examiner rejected claims 1-15, and 21 alleging that the art of transgenics is not a predictable art with respect to transgene behavior, and without evidence to the contrary, transgene expression of different species of transgenic animals varies according to the particular host species. Applicants submit that one skilled in the art would know the correct regulatory elements, i.e. promoters, enhancers, replication origins, etc. for the particular construct/host combination desired. Applicants further submit that the Examiner's relied-upon quote by Mullins, that "a given construct may react very differently from one species to another", does not indicate that the transgene will not actually be expressed from one species to another but rather implies that the level of gene expression may be variable from one species to another. Applicants submit that Wall, Kappel, and Strojek and Wagner also do not disclose that a transgene will not be expressed in particular host due to host effects on the transgene. Applicants submit that varying levels of protein expression by transformed hosts are an accepted consequence of gene therapy in general. The present invention does not guarantee a given level of protein expression for any specific genetic construct but rather the invention is a method for inserting a transgene into a host to produce a transgenic animal. Applicants submit that a skilled artisan would know how to produce a DNA construct to obtain the desired level of expression in a host of their own choosing without undue experimentation. Therefore, applicants respectfully request withdrawal of the rejections of claims 1-15, and 21 under 35 U.S.C. § 112, first paragraph.

The Rejections Under 35 U.S.C. § 103

The Examiner rejected claims 1, and 4-21 as being unpatentable over Lavitrano et al., in view of Kuretake et al. The Examiner alleges that Lavitrano discloses a method of creating a transgenic mouse comprising incubating live, intact mouse sperm with plasmic DNA, fertilizing mouse oocytes with the sperm-DNA complex, transferring the resulting embryos to foster mothers, and analyzing the offspring for the presence of the transgene. The Examiner admits that Lavitrano does not expressly disclose the microinjecting of a complex of DNA and membrane disrupted or demembranated sperm heads into unfertilized mouse oocytes. However, the Examiner alleges that

Kuretake discloses a method of microinjection of sperm having damaged plasma membranes. The Examiner alleges that it would have been obvious to one of ordinary skill in the art to combine the teachings of Lavitrano and Kuretake because disclose that sperm with a damaged plasma membrane increases the fertilization rate by ICSI .

Applicants respectfully traverse this rejection. Applicants respectfully assert that neither Lavitrano nor Kuretake, alone or in combination, teach or suggest incubating an exogenous nucleic acid with a membrane-disrupted sperm head or a demembranated sperm head for a period of time to obtain a sperm head comprising an associated exogenous nucleic acid, and then microinserting the sperm head comprising the associated exogenous nucleic acid into an unfertilized oocyte to form a transgenic fertilized oocyte. At most, Lavitrano teaches fertilization of mouse oocytes with live spermatozoa that have been incubated with plasmid DNA. As the Examiner admits, Lavitrano does not teach or suggest that the spermatozoa are either membrane-disrupted sperm heads or demembranated sperm heads, or that they be microinserted into oocytes. Moreover, Kuretake does not teach or suggest that transgenic embryos and transgenic offspring can be produced by preincubation of such sperm heads with exogenous nucleic acid and microinjection of these heads into oocytes. At most Kuretake teaches that membrane-disrupted or demembranated sperm heads can support development of live offspring. Applicants respectfully submit that one skilled in the art simply would not be motivated to combine the teachings of Lavitrano and Kuretake, because there was previously no evidence that exogenous DNA could be transferred by **dead** spermatozoa. All previous research had been conducted with transfer of exogenous DNA by **live** spermatozoa.

Applicants further point to the statements of Gandolfi above, who refers to applicants' invention as an ***innovative*** approach to the production of transgenic mice through sperm-mediated gene transfer, and to those of Dr. Schatten, whose own work in this area with rhesus monkeys was admitted to be inspired by the publication of the Science article describing the present invention.

In view of the foregoing, applicants respectfully submit that the present invention is patentable and not obvious over Lavitrano and Kuretake, alone or in combination, and respectfully request withdrawal of the rejections under 35 U.S.C. § 103(a).

Conclusion

In view of the foregoing amendments and Remarks, applicants respectfully submit that this application is in condition for allowance, and an early favorable response is respectfully elicited.

Respectfully submitted,

JONES, DAY, REAVIS & POGUE

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"All the News That's Fit to Print"

The New York Times

VOL. CXLIX .... No. 51,745 THURSDAY, DECEMBER 23, 1999

Barak, in a First for Top Israeli, Meets Arafat in the West Bank

Relaxed Setting Seems to Help Solve Land Issue

By DEBORAH SONTAG

RAMALLAH, West Bank, Dec. 22 — Despite speculation that the resumption of Israeli-Syrian talks would derail the Palestinian peace track, an intimate meeting here overnight between Prime Minister Ehud Barak and Yasir Arafat apparently made significant progress.

It was the first time an Israeli prime minister had ventured into a Palestinian-controlled city for a peace meeting, which in itself broke a barrier in the relationship. And the setting was very casual: Mr. Barak and Mr. Arafat met in the elegant home of the Palestinian leader's second in command, Mahmoud Abbas, better known as Abu Mazen, his revolutionary name.

Inside the villa, the two leaders talked for three hours, from late Tuesday until early today, gathered with associates around a banquet of Arabic salads and meat dishes. The security, both Israeli and Palestinian, was tight, but the meeting and its location had been kept secret and there were no disruptions.

After the meeting, both sides said two deadlocked issues that have bedeviled progress were essentially resolved. An Israeli transfer of 5 percent of the West Bank, which has been delayed for five weeks, is now likely to take place within a week, both sides said. And the Israelis are also expected to release more prisoners as a gesture for Ramadan, the Islamic holy month, which ends early next month.

The details were left to negotiators who did not attend the talks, and Saeb Erekat, a senior Palestinian negotiator, had left an early evening session in Jerusalem declaring that not a single issue had been resolved.

It was unclear whether Mr. Erekat was sincerely downbeat or adopting a tough posture. His stance contrasted with the comments from two senior Palestinians who attended the late-night meeting. Exuding confidence, one of them, Nabil Abu Rudeineh, a spokesman for Mr. Arafat, said early today that the Palestinians would "witness results in the very near future."

Another senior Palestinian had suggested that the Palestinians, who

Scientists Place Jellyfish Genes Into Monkeys

By GINA KOLATA

Scientists in Oregon report today that they have installed jellyfish genes in monkey embryos, using a technique that might eventually be

'Beautiful Sight Out Here' . . .

That's what John M. Grunsfeld had to say as he embarked on the first spacewalk c with another astronaut, to begin replacing gyroscopes on the Hubble Space Telesc

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Another senior Palestinian had suggested that the Palestinians, who had held up the transfer of land because it is largely unpopulated desert, were persuaded to accept the original offer.

"Ah, we still have two other redeployments," the official said, referring to land transfers, the first scheduled for January. The official suggested that the Palestinians had been given assurances that more populated territory would be included in the

Continued on Page A12

Scientists Place Jellyfish Genes Into Monkeys

By GINA KOLATA

Scientists in Oregon report today that they have installed jellyfish genes in monkey embryos, using a technique that might eventually be used to create monkeys with added human genes.

Such a technique would allow those genes to be studied for the development of treatments for human diseases.

But the scientists say they are acutely aware that their research raises a host of troubling questions about reproductive technology.

If scientists can add genes to monkey embryos, it should be possible to add genes to human embryos, raising the emotionally fraught issue of human genetic engineering.

"These are matters that need to be discussed," said Dr. Barry Zirkin, who leads the division of reproductive biology at the Johns Hopkins University School of Public Health in Baltimore, who was not connected with the Oregon research.

What stands out, some experts said, is how simple the method is. If it is refined to be highly effective in monkeys, it could be just a short step to using it to add genes to human embryos. Some genes might correct diseases or prevent them — like an AIDS resistance gene, or one that might make a person less susceptible to Alzheimer's disease. Adding such genes might be akin to vaccinating a child. The addition of other genes might be more problematic. Dr. Inder Verma, a molecular biolo-

Continued on Page A20

Algerian Is Indicted; Warning by Clinton

A 32-year-old Algerian man pleaded not guilty to five counts in a federal indictment stemming from his arrest last week near Seattle after customs agents said they found a cache of bomb-making components in his rental car, moments after he drove off a ferry from Canada.

The incident has led to an unusual number of security advisories, including a warning by President Clinton to Americans to be watchful during the coming holidays. Other officials also urged caution but said there was no specific information about a terrorist attack.

Articles, Page A20.

'Beautiful Sight Out Here'

That's what John M. Grunsfeld had t
with another astronaut, to begin repl

Web Sites Bloo

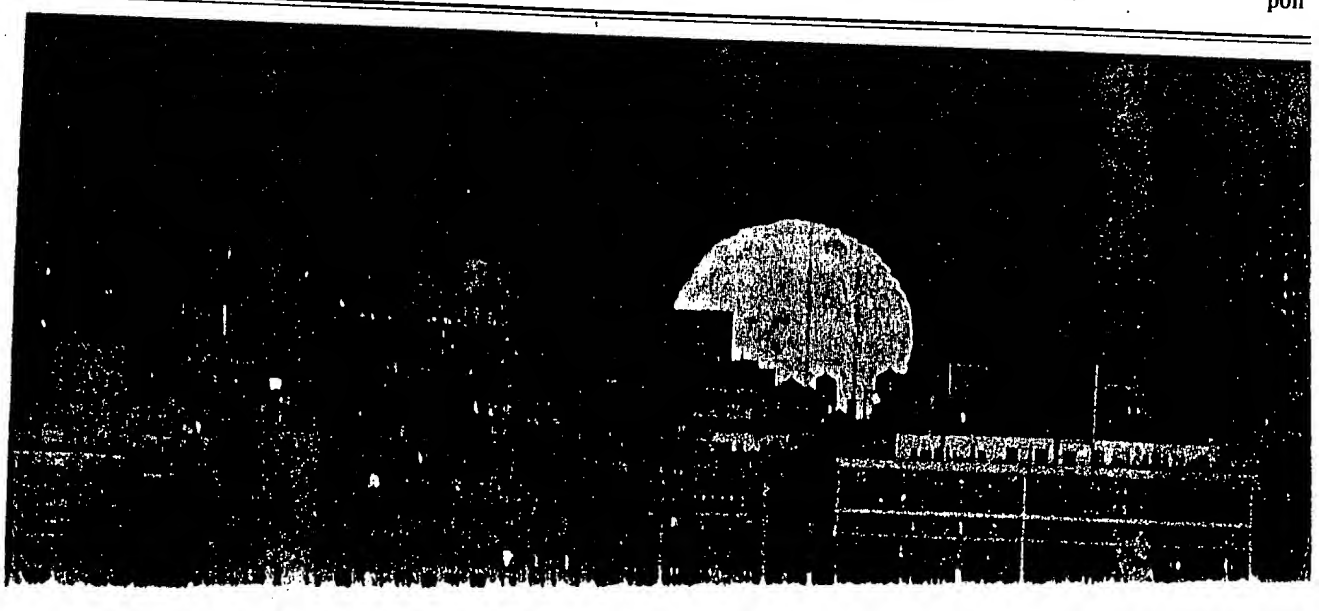
By ELISABETH ROSENTHAL

BEIJING, Dec. 22 — On a recent evening, Zhang Aijing sat at her terminal at the Internet division of People's Daily, watching comments drop from cyberspace into the Communist Party newspaper's chat room.

Ms. Zhang is an "editor" assigned to "anchor" discussions, and her responsibilities include weeding out comments overly critical of the party. With a click of her mouse, ugly thoughts disappear.

She had just posted a notice on the site about a Western reporter's visit and was watching intently to see what came in. After all, unpredictable things happen here, where party propaganda meets cyberspace and, as if to underscore that point, a screen saver flashes smiling images of Diana, Princess of Wales, her wedding and Prince William nearby.

"The U.S. is a killer — we can't forget May 8," comes the first reply, referring to the day last spring when NATO forces bombed the Chinese Embassy in Belgrade. Most Chinese reject NATO's explanation that the attack was an accident. More angry comments tumble in.



we are doing everything we possibly can."

"This is not a terrorism indictment."

But she did issue an unusual plea

wanted on other theft charges in the province of Quebec.

Jellyfish Genes Are Transferred to Monkeys

Continued From Page A1

gist at the Salk Institute in San Diego who also was not connected with the work, gave the example of a gene like one for the glutamate receptor protein in the brain, which may improve memory.

"Suppose you put in an extra glutamate receptor and turn it on before giving a test to a monkey," Dr. Verma said. He said it would be interesting to know whether the monkeys' memories improved.

What if it worked and then humans wanted that gene? "If something can be done, people will do it," Dr. Verma replied, though he added that he was comforted by the thought that there would be very few people who could afford such genetic engineering, and even fewer who would want it.

The current study being published today in the journal Molecular Human Reproduction, is more of a demonstration of feasibility, experts said. To add genes to monkey embryos, Dr. Gerald Schatten of the Oregon Primate Research Center at Oregon Health Sciences University in Beaverton, and his colleagues began by mixing jellyfish genes with sperm cells from rhesus monkeys. The genes stuck naturally to the outside of the sperm cells.

Then the scientists tried to use those sperm to fertilize eggs, asking whether the added jellyfish genes became part of the developing embryos. The gene encodes instructions for a protein that gives jellyfish a green glow. If it got into the monkey embryos and functioned, the embryo cells would glow green under a fluorescent light.

In one set of experiments, the scientists fertilized about 20 monkey eggs in vitro, mixing sperm and eggs in a petri dish. With that method, which mimics natural fertilization, the sperm swims to the egg and discards its protein coat as it enters. The extra gene was discarded with the coat as the sperm penetrated the egg. Only the sperm's genetic material got in.

The scientists also fertilized 81 eggs by directly injecting them with the gene-containing sperm. Then, they report, the jellyfish genes entered the eggs with the sperm. The consequence was clear when the scientists shined a fluorescent light on the embryos. "More than a third of the embryos fluoresced," Dr. Schatten said.

Using seven of the monkey embryos created by direct sperm injection into eggs, Dr. Schatten and his colleagues tried to create pregnancies and got a set of stillborn twins and one live male monkey.

The scientists have not found evidence that the gene was incorporated into any of the monkeys' cells. But, they and others say, they have no doubt that the method will work, because it was recently shown to work in mice.

Earlier this year, in the journal Science, Dr. Ryuzo Yanagimachi at the University of Hawaii and his colleagues reported that they had mixed the same jellyfish gene with mouse sperm, injected the sperm into mouse eggs and created pregnancies. They reported that 11 of the

An experiment with monkeys offers hope for treating disease in humans.

57 mice that were born had the jellyfish gene: when they examined cells from the animals' tail tips under a fluorescent light, the cells glowed green.

Dr. Yanagimachi said that while he was disappointed that the first monkeys did not have the added gene, "this is just a matter of time before it can be done."

Monkeys, Dr. Schatten said, can be invaluable in studies of human disorders.

"When you think of their value as a disease model or for understanding cognition or mental disorders or all sorts of diseases and disorders that appear in people, this is a very small first step," he said.

But, he added, a darker side of the work is the questions it raises about a popular fertility treatment.

The sperm injection method is a standby in fertility clinics, where it is used 10,000 to 20,000 times a year in about 200 American clinics. Discovered by accident less than a decade ago, the method, called intracytoplasmic sperm injection, or ICSI, revolutionized the treatment of male infertility. It allowed men whose sperm do not move, men whose sperm cannot penetrate an egg, and men whose sperm are not even ejaculated, to become fathers.

But ICSI (pronounced ICK-see) puts whole sperm, along with whatever may be attached to them, into egg cells. Dr. Schatten noted that viruses can attach themselves to sperm and no amount of rinsing can get them off. With ICSI, these viruses would be injected directly into eggs where, his work shows, their genes could start to function. And while there is no proof so far that babies born from ICSI procedures have unusual diseases, the prospect has troubled some scientists.

"It's a significant concern," said Dr. Peter N. Schlegel, a specialist in male infertility at the Weill Medical College of Cornell University. But he noted that most of the monkey embryos did not take up the genes that Dr. Schatten attached to the sperm.

Dr. Paul Turek, a specialist in male infertility at the University of California in San Francisco, says he will add the new information from the monkey experiments when he is counseling patients who are considering ICSI. "They can decide whether they want to go forward or not," he said.

But Dr. Turek added that while he would continue with ICSI for those who want it, he would not give a blanket recommendation to everything that becomes technologically possible just because there is a market for it.

"I fall back on the fact that I am an ethical religious man," he said. As such, he said, he continually questions where the boundaries lie.

"People think technology is forging way ahead of biology, ethics, and common sense," Dr. Turek went on. "All of us think about this all the time. All of the clinicians wonder what we are doing."